Original Article

A Retrospective Study on Serologic Weak D Phenotype Among Rh-Negative Blood Donors Undergoing Indirect Antiglobulin Testing at a Tertiary Care Centre

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Abstract

Background: The Rh blood group system is highly significant in transfusion medicine, with the D antigen being the most immunogenic. Weak D, formerly termed Du, represents a quantitative reduction in D antigen expression and may lead to alloimmunization if not correctly identified. Determining the prevalence of serologic weak D among Rh negative donors is essential to enhance transfusion safety. The aim is to determine the prevalence of serologic weak D phenotype among Rh negative blood donors at a tertiary care centre. **Material and Methods:** This retrospective study was conducted over a three-year period from 2022 to 2024 at a tertiary care blood centre. A total of 10,653 blood donations were recorded. Of these, 609 donors were found to be Rh negative. Weak D testing was performed using the indirect antiglobulin test method with monoclonal Anti D reagents. Agglutination after the addition of anti-human globulin was considered confirmatory for weak D positivity. Prevalence was calculated as a percentage of Rh negative donors. **Results:** Among the 10,653 donors, 609 (5.72 %) were Rh negative and 10,043 (94.28 %) were Rh positive. Of the 609 Rh negative donors, 18 were confirmed as weak D positive, yielding a prevalence of 2.96 %. Weak D positivity was found in all ABO blood groups and highest proportion was observed in A negative donor (5.52 %). **Conclusion:** The prevalence of serologic weak D among Rh negative donors in our centre was 2.96 %. Routine weak D testing in Rh negative donors is essential to prevent misclassification and reduce the risk of alloimmunization, thereby ensuring safer transfusion practices.

Keywords: Weak D; Rh-negative donors; Indirect antiglobulin test; Blood transfusion; Alloimmunization.

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Introduction

The Rhesus blood group system was first described in 1939 by Levine and Stetson, who reported a case of hemolytic transfusion reaction caused by an antibody that agglutinated the red blood cells of nearly 85 percent of ABO compatible donors. This landmark observation, following Landsteiner's discovery of the ABO blood group system, represented a major advance in immunohematology and established the clinical relevance of blood group antigens beyond ABO.[1] Since then, the Rh system has been recognized as one of the most complex and clinically significant blood group systems in transfusion medicine.

More than 50 antigens have been identified within the Rh system, of which D, C, c, E and e are the most clinically important. Among these, the D antigen is the most immunogenic and is responsible for the majority of clinically significant Rh alloantibodies. Individuals are categorized as Rh positive or Rh negative based on the presence or absence of the D antigen on the red cell surface. Unlike the ABO system, anti D antibodies are not naturally occurring but are formed following exposure to D positive red cells through transfusion or fetomaternal hemorrhage. Anti D antibodies can cause destruction of red blood cells during blood transfusion and can also lead to serious problems in newborn babies, known as hemolytic disease of the fetus and newborn. Therefore, correct Rh blood group testing is very important in blood transfusion and during pregnancy care.[2]

Rh antigen expression is regulated by RHD and RHCE genes which are located on chromosome.[1] Variations in the RHD gene account for different Rh D phenotypes, including complete absence of D antigen, weak expression of D antigen and partial D variants. The weak D phenotype, previously called Du, means that the D antigen is present on red blood cells but in a smaller amount than normal. Because of this reduced expression, the

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cells may not show clumping during routine immediate spin testing. However, they can show a reaction after incubation at 37 °C and after adding antihuman globulin during the indirect antiglobulin test.[3,4]

The clinical significance of weak D is related to its ability to cause alloimmunization. If blood from a donor with weak D is transfused to a truly Rh negative recipient, the recipient may develop anti D antibodies. To avoid this risk, donors with weak D are considered Rh positive so their blood is not transfused to Rh negative patients. In contrast, individuals with weak D who require transfusion are generally managed as Rh negative, particularly women of childbearing age, to minimize the chance of developing anti D antibodies.[5,6]

The prevalence of weak D differs among various populations and ethnic groups, mainly due to differences in genetic background and regional diversity.[7] Several Indian studies have demonstrated variable frequencies of weak D among Rh negative donors, emphasizing the need for region specific data to guide transfusion policies. Determining the prevalence of serologic weak D in Rh negative blood donors in a tertiary care centre is therefore essential to strengthen transfusion safety practices and reduce the risk of alloimmunization.

Materials and Methods

Study Design and Setting: This retrospective study was conducted over a period of three years from 2022 to 2024 at a tertiary care blood centre. During the study period, a total of 15,783 blood donations were recorded and analyzed from the blood bank database.

Study Population: Among total 15,783 blood donations, 1,290 donors were identified as Rh negative on routine forward grouping and were included in the study population. All donor samples identified as Rh negative were included in the study. Samples typed as Rh positive on routine testing were excluded.

Serologic Testing for Weak D: All samples that were initially typed as Rh negative were further tested for weak D using the indirect antiglobulin test. Weak D testing was carried out when no agglutination was seen with Anti D during routine immediate spin testing.

Reagents and Materials: The reagents used for testing included blend IgG and IgM Monoclonal Anti D, anti-human globulin reagent, IgG coated control red cells, and reagent red cells. The procedure was carried out using glass test tubes, glass pipettes, a table top centrifuge, and a test tube stand.

Procedure: One drop of a two to four percent suspension of test red cells was prepared in a pre labeled test tube. Two drops IgG and IgM Anti D reagent were added to the test tube and mixed thoroughly. The mixture was then incubated at 37 °C for 30 to 60 minutes and then examined for agglutination. If agglutination was observed, the sample was reported as weak D positive. If no agglutination was seen, the red cells were washed 3-4 times with normal saline. The supernatant was then completely discarded after the final wash. Two drops of anti-human globulin reagent were then added and mixed gently. This is followed by centrifugation at 1000 revolutions per minute for one minute. The cell button was resuspended and checked again for agglutination. All negative results were confirmed by adding IgG sensitized control red cells and recentrifuging to ensure the validity of the test.

Interpretation of Results: Agglutination after addition of anti-human globulin reagent was interpreted as weak D positive. Absence of agglutination indicated weak D negative status. In negative reactions, agglutination after addition of control cells confirmed a valid test. Absence of agglutination with control cells rendered the test invalid and the procedure was repeated.

Statistical Analysis: The prevalence of weak D positivity was calculated as a percentage of the total Rh negative donors.

Results

A total of 10,653 blood donors were recorded during the study period of three years. The highest number of donations was observed in year 2023 with 3,817 donors. This was followed by 2024 with 3,499 donors and 2022 with 3,337 donors. Most of donors were Rh positive in all three years. In 2022, 3,139 of 3,337 donors (94.07 %) were Rh positive, while 198 (5.93 %) were Rh negative. In 2023, 3,604 donors (94.42 %) were Rh positive and 213 (5.58 %) were Rh negative. Similarly, in 2024, 3,300 donors (94.34 %) were Rh positive and 198 (5.66 %) were Rh negative. Overall, of the 10,653 donors, 10,043 (94.28 %) were Rh positive and 609 (5.72 %) were Rh negative. The Rh distribution remained consistent across the study period. Rh positive donors forming approximately 94 % of the donor population each year and Rh negative donors accounting for about 5 to 6 %, indicating a stable pattern of Rh distribution [Table 1].

Table 1: Year wise Blood Collection and Rh Distribution.

Year	Total Donors	Rh Positive n (%)	Rh Negative n (%)
2022	3337	3139 (94.07%)	198 (5.93%)
2023	3817	3604 (94.42%)	213 (5.58%)
2024	3499	3300 (94.34%)	198 (5.66%)
Total	10,653	10,043 (94.28%)	609 (5.72%)

Among the 609 Rh negative donors included in the study, O negative was the most common blood group, comprising 217 donors (35.63 %). This was followed by B negative with 194 donors (31.86 %) and A negative with 145 donors (23.81 %). AB negative was the least common blood group, accounting for 53 donors (8.70 %). The distribution demonstrates that O negative and B negative together constituted more than two thirds of the Rh negative donor population. In contrast, AB negative represented less than one tenth of Rh negative donors. Overall, the findings indicate that O negative is the predominant ABO blood group among Rh negative donors in this population, whereas AB negative is comparatively rare [Table 2].

Table 2: Distribution of ABO Blood Groups Among Rh Negative Donors (n = 609).

ABO Group	Number	Percentage (%)
A negative	145	23.81%
B negative	194	31.86%
AB negative	53	8.70%
O negative	217	35.63%
Total	609	100%

Among the 609 Rh negative donors included in the study, 18 were identified as weak D positive, resulting in a prevalence of 2.96 %, while 591 donors (97.04 %) were confirmed as weak D negative. These results show that nearly 3 % of donors who were initially typed as Rh negative on routine testing exhibited serologic weak D positivity when further evaluated using the indirect antiglobulin test. Although most Rh negative donors were truly D antigen negative, a small but clinically important proportion showed weak expression of the D antigen. Identifying weak D in Rh negative donors is essential in transfusion practice, as failure to detect it may lead to incorrect Rh classification and increase the risk of alloimmunization in truly Rh negative recipients [Table 3].

Table 3: Prevalence of Weak D Among Rh Negative Donors.

Parameter	Number	Percentage (%)
Weak D positive	18	2.96%
Weak D negative	591	97.04%
Total Rh negative	609	100%

Among 609 Rh negative donors, weak D positivity was detected in all ABO blood groups. The highest frequency was observed among A negative donors in which 8 of 145 individuals (5.52 %) were weak D positive. This was followed by AB negative donors in which 2 of 53 individuals (3.77 %). In B negative donors, 4 of 194 individuals (2.06 %) showed weak D positivity. The lowest frequency was noted in O negative donors, with 4 of 217 individuals (1.84 %). Overall, 18 of 609 Rh negative donors (2.96 %) were confirmed as weak D positive. Although weak D was present across all ABO groups, it was relatively more common among A negative donors in this population. These findings emphasize the need to perform weak D testing in all Rh negative donors, regardless of ABO type, to ensure accurate Rh classification and improve transfusion safety [Table 4].

Table 4: Distribution of Weak D According to ABO Blood Groups.

ABO Group	Rh Negative	Weak D Positive	Percentage within Group (%)
A	145	8	5.52%
B	194	4	2.06%
AB	53	2	3.77%
O	217	4	1.84%
Total	609	18	2.96%

Discussion

The Rh blood group system remains one of the most clinically significant systems in transfusion medicine because of the highly immunogenic nature of the D antigen. Although routine Rh typing is performed using the immediate spin method, this technique may fail to detect weak expression of the D antigen. The weak D phenotype, first described by Stratton in 1946, refers to a reduced expression of the D antigen on red blood cells and cannot always be detected by routine testing. Accurate identification requires the use of the indirect antiglobulin test. Detecting weak D is important in blood banking practice to prevent alloimmunization and reduce the risk of transfusion related complications.[8]

In the present study, a total of 10,653 blood donors were evaluated over a three-year period. Among them, 609 donors (5.72 %) were Rh negative. Further testing with the indirect antiglobulin test identified 18 donors as weak D positive, resulting in a prevalence of 2.96 % among Rh negative donors.

The prevalence observed in our study is comparatively higher than that reported in several Indian studies. Akriti et al. from Bengaluru documented a weak D prevalence of 1.09 % among Rh negative donors.[8] Krishna et al. in Tirupati reported a frequency of 1.04 %.[9] Singh et al. from Lucknow identified weak D positivity in 1.11 % of Rh negative individuals.[7] Brar et al. observed a prevalence of 1.51 % in the Andaman and Nicobar Islands.[1] Gundrajukuppam et al. in Tirupati demonstrated a lower prevalence of 0.756 % 10. Kanwar et al. from Moradabad reported weak D positivity in 0.19 % of cases.[6] The study by Srivastava et al. reported a low prevalence of 0.027 % in Maharashtra.[11] The differences in the findings of various studies could be explained by regional genetic variability, ethnic heterogeneity, differences in sample size, and variations in serologic testing methods or reagent sensitivity.

Weak D antigen is immunogenic and has the potential to produce alloimmunization if transfused to truly Rh negative recipients.[6] Failure to detect weak D donors may result in incorrect Rh classification. This increases the risk of hemolytic transfusion reactions and sensitization. In the present study, weak D positivity was identified across all ABO blood groups. This highlight the importance of performing weak D testing in every donor initially typed as Rh negative, regardless of ABO type.

These findings highlight the importance of standardized testing protocols in blood banks. Proper identification of variant D phenotypes and correct labeling of donor units are necessary to ensure safe transfusions. Developing and implementing national guidelines for uniform D antigen testing in both donors and recipients would further reduce transfusion related complications. This will help in improving the overall quality and safety of blood transfusion services.[12]

This study provides region specific information on the prevalence of serologic weak D among blood donors over a three-year period at a tertiary care centre, with all Rh negative samples systematically tested using the indirect antiglobulin test. However, since it was a retrospective study conducted at a single centre, the results may not be fully generalizable to other populations or regions. Molecular genotyping was not performed, so differentiation of weak D and partial D variants was not possible. Future multicentric studies incorporating molecular techniques are recommended. Routine weak D testing in all Rh negative donors and implementation of standardized national transfusion guidelines are essential to enhance transfusion safety and prevent alloimmunization.

Conclusion

The present study demonstrated that the prevalence of serologic weak D among Rh negative blood donors in our tertiary care centre was 2.96 %. Although relatively infrequent, weak D represents a clinically significant entity that may lead to misclassification of Rh status if not properly identified. Routine testing of all Rh negative donors using the indirect antiglobulin test is essential to ensure accurate Rh typing and to minimize the risk of alloimmunization in truly Rh negative recipients. Strengthening standardized testing protocols and adherence to transfusion guidelines will further enhance the safety and effectiveness of blood transfusion practices.

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Conflicts of interest

There are no conflicts of interest.

References

1. Brar RK, Shaiji P, Sehgal S. Testing for weak D Antigen: Spectrum and its applied role in rhesus-negative transfusions in Andaman and Nicobar Islands. Tzu Chi Medical Journal. 2020;32(2):167-70.
2. Patel MS, Kakadiya SR, Shah JM. A Study on Weak D Prevalence among Blood Donors and Patients. National Journal of Integrated Research in Medicine. 2017;8(2).
3. Matzhold EM, Bemelmans M, Polin H, Körmöczi GF, Schönbacher M, Wagner T. Characterization of novel RHD allele variants and their implications for routine blood group diagnostics. Biomedicines. 2024;12(2):456.
4. Avent ND, Reid ME. The Rh blood group system: a review. Blood, The Journal of the American Society of Hematology. 2000;95(2):375-87.
5. Sandler SG, Chen LN, Flegel WA. Serological weak D phenotypes: a review and guidance for interpreting the RhD blood type using the RHD genotype. British journal of haematology. 2017;179(1):10-9.
6. Kanwar R, Awasthi S, Dutta S. Detection of Weak Rh D (DU) Phenotype among Blood Donors. Annals of International Medical and Dental Research. 2018;4(2):31-3.
7. Singh A, Solanki A, Agarwal D, Chandra T. Prevalance of serologic weak D in Rh D negative blood donors in India: Immunohematological problems & recommendations for donors. Panacea Journal of Medical Sciences. 2023;12(3):686-9.
8. Singh A, Belagatti S, Simeon B, Budha R. Prevalence of weak D antigen in Rh negative blood group: an experience at a tertiary blood centre in Bengaluru, southern India. Natl J Lab Med. 2023;6:14-6.
9. Krishna GD, Babu KS, Arun R, Jothibai D. A study on Rh incompatibility and frequency of weak D among blood donors and patients at a tertiary care referral teaching hospital in Tirupati, Andhra Pradesh. Journal of Clinical and Scientific Research. 2015;4(4):281-4.
10. Gundrajukuppam DK, Vijaya SBK, Rajendran A, Sarella JD. Prevalence of principal Rh blood group antigens in blood donors at the blood bank of a tertiary care hospital in Southern India. Journal of clinical and diagnostic research: JCDR. 2016;10(5):EC07.
11. Srivastava AR, Dhote SW, Singh I. A retrospective study on the prevalence of weak D antigen (Du) in a blood bank in a tertiary care hospital in Maharashtra, India. MGM Journal of Medical Sciences. 2021;8(4):410-4.
12. Srivastava RK, Prasad N, Halder D. Study of Prevalence of Weak D Antigen (D) Amongst Supposed Rh Negative Blood Donors in a Tertiary Care Hospital of Jharkhand. International Journal of Scientific Research. 2018;7(8):69-70.